Development of an Ethanol Yield Procedure for Dry-Grind Corn Processing

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ABSTRACT

In 2008, the United States produced ethanol at a rate of 39.5 billion L/year; an additional 8.5 billion L/year capacity was under construction. Kernel composition and physical properties are not correlated with ethanol yield. A procedure that measured the potential of hybrids to produce ethanol would benefit corn seed companies, corn producers, and ethanol processors. The objective was to develop a laboratory procedure to measure ethanol yield from corn samples and evaluate the developed procedure for accuracy and precision. To determine parameters for routine analyses, effects of mill type, dry solids, and yeast addition were investigated separately followed by effects of fermentation time (T_f), glucoamylase dose, and yeast addition. Measurement of ethanol using HPLC and gravimetric (change in weight due to CO_2 loss) methods were compared. Using the procedure developed, ethanol yields for five diverse hybrids (dent, waxy, white, high oil, and high amylose) were measured. Effects of mill type, dry solids, T_f, glucoamylase dose, and yeast addition were significant ($P < 0.05$). The gravimetric method estimated higher yields (428 ± 10 L/tonne) than HPLC (405 ± 15 L/tonne) and had a higher level of precision. Both methods had coefficients of variations of <4% and gave similar conclusions. In the final procedure, we used corn (25 g/batch) liquefied with α-amylase (60 min at 90°C) in 75 mL of distilled water. Simultaneous saccharification and fermentation was used (64 hr at 32°C) with glucoamylase and yeast. Gravimetric and HPLC methods measured differences in ethanol yield for the five hybrids (158–435 L/tonne). The method is suitable for routine testing of ethanol yield potential and as a reference method for verifying more rapid measurement techniques.

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In 2008, the United States increased fuel ethanol production to a rate of 39.5 billion L/year; an additional 8.5 billion L/year was under construction (RFA 2008). Ethanol has been used as an fuel oxygenate, replacing methyl tertiary butyl ether (MTBE), and as a gasoline extender to decrease the demand for imported petroleum (Shapouri et al 2003).

By identifying corn hybrids with potential for improved ethanol yields, processing of corn into ethanol can have higher efficiency. A procedure to predict accurately hybrid potential would benefit corn genetics companies, ethanol processors, as well as corn producers. A limited number of investigators (Dien et al 2002; Haefele et al 2003; Singh and Graeber 2005) reported the influence of corn hybrid selection on ethanol production in a laboratory-scale process (≈300 mL volume) and concluded that ethanol yields were not dependent exclusively on starch content. Naidu et al (2007) concluded particle size affected ethanol yield; finer particles were associated with higher ethanol yields. Most studies reported that further research was needed to determine experimental variables that influence ethanol yield.

Correlation of ethanol yield potential with other processing parameters has been reported. Dien et al (2002) used a procedure to measure ethanol yield potential and measured extractable starch using a laboratory-scale wet-milling procedure (Eckhoff et al 1996). Using five corn hybrids, they found starch extractability was not correlated highly ($R^2 = 0.42$) with ethanol fermentability. Pruiett (2002; and unpublished data) studied 18 corn hybrids using wet milling and an abbreviated dry-grind procedure that determined total glucose concentrations; these data were compared with extractable starch yields. Glucose and extractable starch yields were not correlated ($R^2 = 0.05$). Singh and Graeber (2005) determined ethanol yields and extractable starch yields for 18 hybrids grown in four locations. They reported no correlation between final ethanol concentration and extractable starch yield ($R^2 = 0.0038$) or total starch content ($R^2 = 0.0001$). Haefele et al (2004) conducted laboratory analysis of corn from a broad genetic sample set of 26 hybrids. They measured ethanol yield potential as carbon dioxide loss per unit mass of corn solids and found low correlation between total starch content and ethanol yield ($R^2 = 0.62$).

Low correlation of composition with ethanol production characteristics was not limited to corn. Zhan et al (2003) used eight sorghum cultivars and studied the effect of cultivar and growing conditions on ethanol production. They used a dry-grind process and found that total starch contents were not correlated with ethanol yields ($R^2 = 0.25$); protein content was correlated more closely with ethanol yields ($R^2 = 0.71$). They concluded that genetics and growing conditions (location) for grain sorghum had an effect on ethanol yield. Therefore, fermentability and ethanol yield must be measured directly, as opposed to using a correlation with composition or extractable starch yields.

With growth of the biofuels industry in the United States, a standardized method is needed that could estimate ethanol yield accurately with documented precision. Rapid methods of ethanol yield measurement such as near-infrared reflectance and transmittance (NIR and NIT, respectively) indicate promise. However, the bases for these methods are data created by reference methods. Rapid methods also require continuous verification and calibration with the reference method. This has contributed to the need for a standardized ethanol yield procedure.

Previous workers have studied fuel ethanol production from feedstocks other than corn (Ingledew et al 1995; Thomas and Ingledew 1995; Sosulski et al 1997; Wang et al 1997; Zhan et al 2003). Taylor et al (2001) used 1 kg of corn samples and a sequential saccharification and fermentation process. Dien et al (2002) and VanCauwenberge et al (1982) used sequential saccharification and fermentation, methods not used in industry. They also used batch sizes of 300 and 560 mL, respectively. Naidu et al (2007) developed a 500-g procedure for corn; a smaller scale procedure is needed that uses enzymes, yeast, and conditions similar to industry practice. Such a procedure would be helpful because genetic material may have limited availability and larger sample size reduces laboratory capacity. Few investigators have studied ethanol concentrations in fermentation broths and compared them with inexpensive measurement methods such as loss in weight methods.

The objectives were to 1) develop procedure parameters and observe the effects on measurement of ethanol yield, and 2) verify precision and accuracy of a laboratory procedure to measure ethanol yield from corn samples.

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MATERIALS AND METHODS

Procedure Development

Corn. We used a regular dent, non Bt corn hybrid (34N43, Pioneer Hi-Bred International, Johnston, IA) grown at the Agricultural and Biological Engineering Research Farm at the University of Illinois in 2003. Processing and testing procedures were conducted in 2004. Corn was stored in sealed bags at 4°C until processing. Test weight (bulk density) (Approved Method 84-10, AACC International 2000) was determined to be 58.4 lb/bu (1,040 kg/tonne); 1,000 kernel weight was 296 g. Composition of whole corn was 12.4% (wb) moisture, 8.1% crude protein, 69.1% starch, and 3.9% (db) crude fat (Analab, Fulton, IL).

Particle size reduction. Except during the milling treatment experiment, corn samples (500 g) were ground using a hammermill (model MF 10, IKA, Werke, Germany) equipped with a round-hole screen, 3 mm in diameter, operating at a grind rate of 120 ± 10 g/min and 4,500 rpm. To determine moisture content of ground samples, 25–30 g were dried in a convection air oven at 135°C for 2 hr (Approved Method 44-18, AACC International 2000). Moisture contents were determined in triplicate.

α-Amylase and liquefaction. α-Amylase (food grade, Spezyme Fred, lot number 107-02285-003, Genencor, Beloit, WI) was added (27 μL/flask or 108 μL/100 g of corn) with an electronic pipette (model Repeater Pro, Eppendorf, Hamburg, Germany) after the slurry was adjusted to pH 6.0–6.5 with dilute NaOH solution. Flasks with slurry and α-amylase were placed into a water bath at 90°C. Flasks remained in the bath for 60 min after reaching 90°C. To avoid caking of the corn slurry, flasks were swirled vigorously by hand during the first 5 min and then every 20 min. After 60 min, flasks were removed and cooled to 40°C. In a preliminary study, we used 1× and 2× the manufacturer's recommended dose of α-amylase and found no effect on ethanol yield (data not shown).

Glucoamylase dose, yeast addition, and simultaneous saccharification and fermentation. Once the liquefied slurry was adjusted to 40°C, it was adjusted to pH 4.5–5.0 by adding 325 μL of concentrated HCl solution (20% v/v). A yeast nutrient, (400 μL/flask of 12 g of 99.9% $(NH_4)_2SO_4$/100 mL of distilled water) and 80 μL of glucoamylase/100 g of corn (food grade, GC 480, lot number 101-01292-016, Genencor, Beloit, WI) were added to each flask. Yeast inoculum (Fleischmann's, Fenton, MO) was added at a rate of 4.2 mL or 0.3 g of yeast/flask. Flasks were capped with a rubber stopper and swirled to suspend particles. Initial flask weight, liquefied corn slurry, enzyme, and yeast were measured. Flasks were placed in an incubator shaker (model C24, New Brunswick Scientific, New Brunswick, NJ) at 150 rpm and 32°C for 64 hr unless noted.

Dry Grinding Treatments

The first step in corn dry-grind processing is particle size reduction, or grinding. The resulting distribution of corn particle sizes affects rate of water penetration, heat transfer, starch gelatinization, enzyme kinetics, and yeast fermentation. Therefore, grinding equipment should give uniform results when milling the same material.

Four mill types were used to prepare corn samples for the dry-grind procedure. Five grinding treatments were used: hammermill (model MF 10, IKA, Werke, Germany), Quaker City (model 4E, The Straub Co., Warminster, PA), Retsch (model SK100/S Spezialstahl, Retsch GmBH, Haan, Germany), Romer (coarse setting; model 2A, Romer Labs, Union, MO) and Romer (fine setting). These mills represented equipment available to laboratories. Corn was ground through each mill and sieved through standard 10, 20, 30, 40, 50, and 60 mesh screens (2.0, 0.84, 0.60, 0.43, 0.30, and 0.25 mm openings, respectively) and pan for 5-min periods using a Ro-Tap shaker (no. 3775, W.S. Tyler Company, Cleveland, OH). Fractions remaining on each screen were weighed and expressed as a percentage of original sample weight. Sufficient corn was milled to conduct three replicate particle size distribution measurements. Particle size distributions were compared with distributions from commercial dry grind facilities (Rausch et al 2005).

Ground corn from each treatment was mixed and five samples (25 g each) per treatment were placed into flasks for fermentation. Flasks were prepared using 75 mL of distilled water, liquefied with amylase, and placed in the fermenter for a 64-hr period. Ethanol yields were estimated by loss in weight method.

Solids Content During Fermentation

The effects of initial solids content in fermentation flasks were investigated. Lower solids levels in the flasks allows easier agitation and suspension of substrate, enzymes, and yeast during fermentation, but results in less CO_2 evolved and smaller changes in weight that must be measured accurately to estimate ethanol yield. Higher solids contents result in more CO_2 evolved and more DDGS to use, if needed, for further study. However, higher solids can make mixing more difficult and result in lower efficiency of starch conversion to ethanol. Initial solids contents of the slurry during fermentation was varied for five treatment levels (20, 25, 30, 35, and 40% solids). Each level was replicated five times. Liquefaction and SSF conditions were the same as described in previous sections.

Yeast Addition

The concentration of yeast was varied to determine sensitivity of ethanol yield to yeast addition and determine whether an optimum yield could be observed. Industrial yeast addition rates are 0.88–1.76 kg/tonne of corn. Yeast (Fleischmann's, Fenton, MO) was added at a relatively broad range of addition levels from 0.5 to 3.0 g of yeast/100 g of corn in 0.5-g increments. Treatments were replicated five times. Mean ethanol yields were determined using the loss in weight method.

Batch Variation, Fermentation Time, Glucoamylase Dose, and Yeast Addition

In industrial fuel ethanol practice, fermentation times (T_f) vary from 48 to 72 hr. In laboratory practice, longer T_f achieve higher conversion rates but also reduce the capacity of the procedure. Other laboratory methods (e.g., Haefele et al 2004) provide excess enzyme, yeast, nutrients, and T_f so that differences observed are presumed due to genetics alone. However, interaction of genetics with restricted (i.e., economical) process input is not well understood; use of high levels of process input may result in unrealistic ethanol yield determinations. Our objective was to observe batch variation and sensitivity of ethanol yields to T_f, glucoamylase dose, and yeast addition. From these results, we would determine the process input that would provide precise ethanol yield determinations balanced with practical considerations of throughput and sample processing.

In a randomized complete block factorial design, four T_f (46, 52, 64, and 72 hr) were observed using the loss in weight method. This allowed using the same batch material for each T_f without altering the broth as would be required for HPLC analyses. Glucoamylase was added at three levels (40, 80, and 160 μL/100 g of corn). Yeast was added at two levels: 0.4 and 1.2 g/100 g of corn. The six enzyme and yeast treatments were replicated four times within each batch (24 flasks/batch); each fermentation using the incubator shaker formed one complete block of enzyme and yeast treatments. Four batches (blocks) were fermented; this design provided variability measurement among batches as well as among treatments. Statistical analyses used the GLM procedure (v.8.02, SAS Institute, Cary, NC) to detect differences among ethanol yield means. ANOVA analysis determined F values and when source effects were significant ($P < 0.05$) yield means were compared using the least significant difference method. Interaction effects also were determined.

Final Procedure

For testing the final procedure, a regular dent corn hybrid (34N43, Pioneer Hi-Bred International, Johnston, IA) was obtained from the Agricultural and Biological Engineering Research Farm at the University of Illinois in 2003. Test weight (bulk density) (Approved Method 84-10, AACC International 2000) was determined to be 1,040 kg/tonne; 1,000 kernel weight was 296 g. Composition of whole corn was 12.4% (wb) moisture, 8.1% crude protein, 69.1% starch, and 3.9% (db) crude fat (Analab, Fulton, IL).

α-Amylase (food grade, lot number 107-02285-003, Spezyme Fred, Genencor, Beloit, WI) was used for liquefaction. Glucoamylase (food grade, lot number 101-01292-016, GC 480, Genencor, Beloit, WI) and active dry yeast (Fleischmann's, Fenton, MO) were used for simultaneous saccharification and fermentation (SSF) process steps.

Sample Preparation

Corn samples (500 g) were ground using a hammermill (model MF 10, IKA, Werke, Germany) equipped with a round-hole screen, 3 mm in diameter, and operating at a grind rate of 120 ± 10 g/min and 4,500 rpm. To determine moisture content of ground sample, 25–30 g were dried in a convection air oven at 135°C for 2 hr (AACC International 2000). Moisture content was determined in triplicate.

Ground samples were mixed and 25 g placed in 125-mL Erlenmeyer flasks. Water (75 mL) was added; pH (model AB15, Accumet, Fisher Scientific Company, Singapore) of the mixture was measured, typically at pH 6.0–6.5. Flasks were capped with rubber stoppers. For gas dissipation, needles (18 ga × 38.1 mm) were inserted into the rubber caps.

Liquefaction and Yeast Inoculation

An 27-μL aliquot of α-amylase/flask (108 μL/100 g of corn) was added with an electronic pipette (model Repeater Pro, Eppendorf, Hamburg, Germany) after the slurry was adjusted to pH 6.0–6.5 with dilute NaOH solution. Flasks with slurry and α-amylase were placed into a water bath at 90°C. Flasks remained in the bath for 60 min after reaching 90°C. To avoid caking of slurry, flasks were swirled vigorously during the first 5 min and then every 20 min. After 60 min, flasks were removed and cooled to 40°C.

Sufficient inoculum for 24 flasks was prepared by mixing 7.2 g of active dry yeast with 100 mL of deionized water and incubating in a water bath at 37°C for 30 min before adding to flasks. The yeast concentration was 1.2 g of dry yeast/100 g of corn solids or 0.3 g of yeast/flask.

Saccharification and Fermentation

Once the liquefied slurry was cooled to 40°C and adjusted to pH 4.5–5.0 by adding 325 μL of concentrated HCl solution (20% v/v). A yeast nutrient (400 μL/flask of 12 g $(NH_4)_2SO_4$ 99.9%/100 mL of distilled water) and glucoamylase (80 μL/100 g of corn) were added. Yeast inoculum was added (4.2 mL or 0.3 g of yeast/flask; 16.8 mL or 1.2 g/100 g of corn, respectively). Flasks were capped and swirled to suspend particles. The initial weight of the flask, liquefied corn slurry, enzyme, and yeast was measured. Flasks were placed in an incubator shaker (model C24, New Brunswick Scientific, New Brunswick, NJ) at 150 rpm and 32°C for 64 hr.

Gravimetric Measurements and HPLC Analyses

Ethanol yields based on weight loss were calculated from weight of corn added for fermentation (db) and net change in weights of the flasks due to fermentation. Four fermentation batches of 24 flasks each were used to determine the final procedure variability. Aqueous ethanol blanks (no corn, enzyme, or yeast) were used to check for potential evaporation errors. Evaporation from flasks during fermentation was negligible. Gravimetric determination of ethanol yields was considered to be the weight difference during fermentation as CO_2 evolved during 64 hr and to be related directly to actual ethanol production by the yeast.

After 64 hr in the fermenter, aliquots (2 mL) of fermenter broth were placed in a plastic centrifuge tubes and centrifuged (model Centra CL3, Thermo IEC, Needham Heights, MA) for 10 min at 4,000 rpm; supernatant was collected in 2-mL plastic vials with screw cap lids and rubber O-ring seals and refrigerated. For HPLC measurements, 10 μL of sample was diluted in 90 μL of deionized water and placed in 1.5-mL HPLC vials and stored at 4°C.

HPLC analyses were performed to determine ethanol concentration (% w/v) using a Finnigan SpectraSystem HPLC system (Thermo Electron, Waltham, MA) equipped with an Aminex HPX-87H column (300 × 7.8 mm, Bio-Rad, Richmond, CA) and an RI150 refractive index detector (Thermo Electron, Waltham, MA). Samples were eluted at 0.6 mL/min with 5 m*M* sulfuric acid at 65°C.

Testing Among Batch Variability and Sensitivity to Unique Hybrids

The variability of the final procedure was measured using four batches of 24 flasks each; ethanol yield was determined by gravimetric and HPLC methods. Statistical analyses followed the GLM procedure (v.8.02, SAS Institute, Cary, NC). For gravimetric and HPLC ethanol yields, we used analysis of variance to compare means from each fermenter batch. Means were compared using the least significant difference method (Fisher LSD) with $P < 0.05$.

Five hybrids (dent, waxy, white, high oil, and high amylose; Wyffels Hybrids, Geneseo, IL) likely to produce differences in ethanol yield were tested using the final procedure. A randomized complete block design with hybrids as treatments and six replications per block were used. Two fermenter batches were used for a total of 60 observations (12 observations/treatment). Mean ethanol yields were compared using the methods described above.

RESULTS AND DISCUSSION

Dry Grinding Treatment

Each milling treatment resulted in unique particle size distributions (Table I). None of the laboratory mills reproduced distributions generated by the larger commercial mills. The commercial sample had larger amounts (11.5%) retained on the largest (10 mesh) screen compared with samples created from laboratory mills (0.1–6.9%). The hammermill had relatively low variabilities (COV 2.2–20.6%) and the Retsch mill had high variabilities (COV 63–236%) for each particle size category relative to other treatments. Particle size distributions varied among milling treatment and COV values were large. However, all but one treatment (Romer coarse) had similar ethanol yields (Table I). The hammermill was used in subsequent experimentation.

Solids Content and Yeast Concentration During Fermentation

Ethanol yields were 353–440 L/tonne (Table II). Lower ethanol yields were observed at 35 and 40% solids; these were attributed to poor mixing during fermentation. A solids content of 30% provided high ethanol yield and was typical of industrial practice (typically 30–32%) (Kelsall and Lyons 2003). However, there was limited supernatant after fermentation, making sample preparations for HPLC analyses difficult. Therefore, a solids content of 25% was selected for subsequent use. No differences were detected in ethanol yield for the yeast concentrations tested (data not shown).

TABLE I
Effects of Milling Treatment on Particle Size Distributions, Variations (COV), and Ethanol Yields

	Hammermill		Quaker City		Romer Fine		Romer Coarse		Retsch		Commercial[c]
Screen[a]	Retained[b]	COV	Retained	COV	Retained	COV	Retained	COV	Retained	COV	Retained
10	0.8	3.5	6.9	19.5	0.1	28.7	2.0	13.8	0.4	63.0	11.5
20	45.7	2.2	53.9	35.8	41.9	2.0	69.1	1.8	42.3	121	56.5
30	19.5	10.7	20.9	46.2	25.5	8.1	10.9	2.3	29.2	78.0	17.5
40	5.6	12.0	2.9	22.3	8.0	38.4	5.3	5.5	18.1	166	8.3
50	11.0	7.3	7.0	49.4	10.7	16.7	4.8	77.9	7.6	117	nd
60	5.3	14.3	1.6	147	7.0	57.6	1.2	150	0.4	90.0	2.6
Pan	12.1	20.6	6.7	21.0	6.8	15.3	6.7	0.6	2.0	236	0.7
Ethanol yields[d]											
gal/bu	2.94		2.97		2.92		2.81		2.91		
L/tonne	438a		443a		435ab		419b		434ab		

[a] Screen sizes are standard U.S. mesh sizes; nd, not determined.
[b] Retained, % material retained on each screen. COV, % standard deviation relative to individual mean.
[c] From Rausch et al (2005); COV was not determined.
[d] Treatments followed by the same letter are not significantly different ($P < 0.05$).

TABLE II
Effect of Solids During Fermentation on Ethanol Yields[a]

	Ethanol Yield	
Fermentation Solids (%)	(gal/bu)	(L/tonne)
20	2.95	440a
25	2.80	417b
30	2.86	426ab
35	2.81	419b
40	2.37	353c

[a] Means followed by the same letter are not significantly different ($P < 0.05$).

TABLE III
Effect of Replicate Fermenter Batch on Ethanol Yields[a]

	Ethanol Yield	
Fermenter Batch	(gal/bu)	(L/tonne)
1	2.79	416a
2	2.79	415a
3	2.63	393b
4	2.61	390b

[a] Means followed by the same letter are not significantly different ($P < 0.05$).

TABLE IV
Effects of Fermentation Time and Yeast Addition on Ethanol Yields[a]

	Yeast Addition	Ethanol Yield		
T_f (hr)[b]	(g/100 g of corn)	(gal/bu)	(L/tonne)	COV (%)
46	0.4	2.51	375e	11.8
46	1.2	2.63	392d	10.7
52	0.4	2.60	388d	10.8
52	1.2	2.71	403c	9.8
64	0.4	2.73	407bc	9.1
64	1.2	2.81	418a	8.3
72	0.4	2.79	416ab	8.2
72	1.2	2.86	426a	7.6

[a] Means followed by the same letter are not significantly different ($P < 0.05$). COV, coefficient of variability.
[b] Fermentation time.

Batch Variation, Fermentation Time, Glucoamylase Dose, and Yeast Addition

There were no interaction effects among batch, T_f, glucoamylase dose, and yeast addition. However, there were differences among batches (Table III). Ethanol yields were 390–416 L/tonne. Based on these results, complete treatment blocks were used for future fermentations.

Longer T_f and higher yeast addition rates increased ethanol yields from 375 to 426 L/tonne (Table IV). Increasing T_f from 46 to 72 hr increased ethanol yields. Higher yeast addition (1.2 g/100 g of corn) at T_f = 64 hr resulted in ethanol yields similar to yields obtained using a lower level of yeast addition at 72 hr. As T_f and yeast addition rates increased, COV of ethanol yield means were reduced from 11.8% to 7.6% (for 46 hr and 0.4 g of yeast/100 g of corn and 72 hr and 1.2 g of yeast/100 g of corn). In selecting T_f and yeast addition rates for the final procedure, COV was monitored when considering highest possible ethanol yields and selecting procedure times and rates similar to commercial scale ethanol production. T_f of 64 hr was chosen for the final procedure because this was more convenient than 72 hr and allowed time during typical working hours to weigh flasks and collect data before and after fermentation.

TABLE V
Effects of Fermentation Time and Glucoamylase Addition on Ethanol Yields[a]

	Glucoamylase Dose	Ethanol Yield		
T_f (hr)[b]	(μL/100 g of corn)	(gal/bu)	(L/tonne)	COV (%)
46	40	2.30	342h	13.4
46	80	2.67	398de	6.4
46	160	2.74	408cd	5.8
52	40	2.40	358g	12.7
52	80	2.76	411cd	5.5
52	160	2.80	417bc	5.2
64	40	2.56	382f	11.4
64	80	2.86	426ab	4.3
64	160	2.88	430ab	4.3
72	40	2.64	393ef	10.5
72	80	2.91	434a	3.8
72	160	2.93	436a	3.8

[a] Means followed by the same letter are not significantly different ($P < 0.05$). COV, coefficient of variability.
[b] Fermentation time.

Longer T_f and increased glucoamylase dosages increased ethanol yields (Table V). At the two higher glucoamylase dosages, ethanol yields were similar for T_f 72 and 64 hr. At each T_f monitored, low glucoamylase dose (0.4 μL/100 g of corn) resulted in lowered ethanol yields. Increased glucoamylase dose reduced COV of yield means for each T_f. In industry, typical doses are 0.8 L/tonne of corn (Graves et al 2007). As glucoamylase dose was reduced, ethanol yield was affected at both yeast addition rates tested (Table VI).

Final Procedure

Ethanol yields among batches produced different yields and a range of 422–432 L/tonne (Tables VII and VIII). HPLC yields were 395–413 L/tonne. HPLC and gravimetric methods had variation within and among batches of <4% relative to the respec-

TABLE VI
Effects of Glucoamylase and Yeast Addition on Ethanol Yield[a]

Yeast Addition (g/100 g of corn)	Glucoamylase Dose (μL/100 g of corn)	Ethanol Yield (gal/bu)	Ethanol Yield (L/tonne)	COV (%)
0.4	40	2.45	365c	14.7
1.2	40	2.50	372c	11.3
0.4	80	2.74	408b	6.6
1.2	80	2.86	426a	4.5
0.4	160	2.78	415b	5.4
1.2	160	2.89	431a	4.6

[a] Means followed by the same letter are not significantly different ($P < 0.05$); COV, coefficient of variability.

TABLE VII
Parameters for Routine Use of Final Procedure

Parameter	Range Tested	Selected Level or Value
Corn grinding	4 mill types, 5 treatments	Hammermill
Solids in fermenter (% total solids)	20–40	25
Liquefaction pH	na	6.0–6.5
α-amylase (μL/100 g of corn)	na	108
Saccharification pH	na	4.5–5.0
Glucoamylase dose(μL/100 g of corn)	40–160	80
Yeast addition (g/100 g of corn)	0.5–3.0	1.2
Fermentation time (hr)	46–72	64

TABLE VIII
Mean Ethanol Yields from Gravimetric and HPLC Methods[a]

Batch	Ethanol Yield – Gravimetric (gal/bu)	Ethanol Yield – Gravimetric (L/tonne)	Ethanol Yield – Gravimetric COV (%)	Ethanol Yield – HPLC (gal/bu)	Ethanol Yield – HPLC (L/tonne)	Ethanol Yield – HPLC COV (%)
1	2.90	432a	1.9	2.74	408ab	2.7
2	2.86	426ab	3.1	2.77	413a	3.5
3	2.85	425ab	2.4	2.76	411a	3.6
4	2.83	422b	2.2	2.65	395b	3.7
Overall	2.87	428	2.4	2.72	405	3.7

[a] Means followed by the same letter are not significantly different ($P < 0.05$); COV, coefficient of variability.

tive overall means. The mean difference between methods was 22 ± 12 L/tonne. Ethanol yields from HPLC measurements (405 ± 15 L/tonne) had larger variations than gravimetric measurements (428 ± 10 L/tonne). The increased measurement error associated with the HPLC may have been associated with sample preparation such as dilution of samples or integration of sugar and ethanol peaks. Dien et al (2002) also observed ethanol yields determined by gravimetric measurements were consistently higher than those determined by HPLC measurements. Ethanol yields using the gravimetric method may reflect weight loss (CO_2 evolution) due to fermentation products other than ethanol. Therefore, it is likely the ethanol yields measured by HPLC were more accurate than those measured gravimetrically.

Using either method for ethanol measurement resulted in the same statistical inferences (Table VII). Differences detected among fermenter batches suggest that each batch should contain several flasks of a standard corn sample. Data from these standard flasks would quantify fluctuations in ethanol yield attributed to procedure variation and facilitate comparison of data among fermenter batches.

Dent, waxy and white hybrids had similar ethanol yields (Table IX). High oil and high amylose hybrids had lower yields. For the high oil hybrid, this was presumed to be due to more oil or germ replacing fermentable starch, while the high amylose hybrid had starch that was not converted to glucose.

TABLE IX
Mean Ethanol Yields for Five Hybrids[a]

Hybrid	Ethanol Yield (gal/bu)	COV (%)
Dent	2.92a	1.8
Waxy	2.85a	3.2
White	2.83a	5.0
High oil	2.47b	3.8
High amylose	1.06c	1.1

[a] Means followed by the same letter are not significantly different ($P < 0.05$); COV, coefficient of variability.

CONCLUSIONS

Five laboratory milling treatments produced particle size distributions with high COV and did not exhibit the same distribution as commercial hammermills. However, all but one of the milling treatments produced similar ethanol yields. Varying initial solids contents of fermentation mash had an effect on ethanol yields; an initial solids content of 25% was chosen.

Certain levels and combinations of T_f, glucoamylase dose, and yeast addition decreased the COV of ethanol yield means. Yeast addition of 1.2 g/100 g of corn and T_f of 64 hr was similar to yeast addition of 0.4 g/100 g of corn and T_f of 72 hr. Glucoamylase dose of 80 or 160 μL/100 g of corn and T_f of 64 hr produced similar ethanol yields to using T_f of 72 hr. Differences among fermentation batches were indicative that standard (known) samples should be included in each fermentation batch, or all batches should include complete treatment groupings (i.e., complete treatment blocks) for experimental design.

Two methods (gravimetric and HPLC) were used to measure ethanol yields; differences in ethanol yields (22 ± 12 L/tonne) were found. The gravimetric method gave consistently higher yields and had a higher level of precision than the HPLC method. Both methods resulted in similar statistical inferences when comparing means.

The final procedure used amylase (108 μL/100 g of corn), glucoamylase (80 μL/100 g of corn), yeast (1.2 g/100 g of corn) and a fermentation time of 64 hr. COV of the final procedure was <4%.

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